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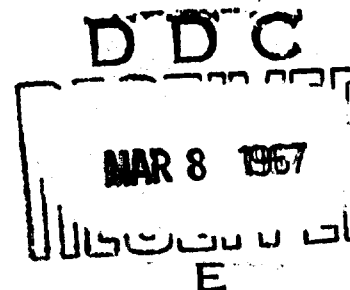
A CONTRIBUTION TO THE STUDY OF THE
TSUTSUGAMUSHI FEVER CAUSATIVE AGENT

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Following is the translation of an article by I. V. Tarasevich in the Russian-language journal Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 3, 1964, pages 11-14.

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Tsutsugamushi fever is one of the rickettsioses not established in the USSR. The incidence and natural foci of tsutsugamushi have been noted in neighboring countries -- China, North Korea, Japan (Yu Eu - Shou et al, 1957; Jackson et al, 1957; Cokes -- cited in Rivers, 1955). In studying typhoid fevers in the Far East, Mill' (1936), Antonov and Nayshtat (1937), and Fedukovich (1938) suggested that tsutsugamushi fever existed in this area. This opinion was seconded by Pavlovskiy (1947).

In recent years, data has been obtained on the geographic variability of the causative agents of tick-borne typhus of North Asia, isolated from the blood of human patients, rat organs, and ticks in the Primor'ye. One of the properties of these strains is close to the causative agent of tsutsugamushi. Thus, Somov et al (1958), Shapiro (1958), and Kulagin et al (1960) established that in sera of experimental animals infected with these strains antibodies to the proteus OX_K predominate, which is characteristic for tsutsugamushi. These assumptions and facts have aroused interest in studying the causative agent of this rickettsiosis.

The strains of Rickettsia tsutsugamushi Gilliam and Karp we studied are among those best known in most of the laboratories of the world. The Gilliam strain was isolated from the blood of an affected physician in Burma (Bengtson, 1945), and the Karp strain was isolated by Derick and Brown in 1947 from the blood of an American soldier infected in New Guinea.

We isolated certain morphological and biological properties of both strains upon passage in white mice, guinea pigs, and chick embryos [See Note].

(NOTE) Previously, we had been convinced that the strains studied did not grow in martenov broth and in agar, the Kit-Tarozzi medium, blood agar, and led to the formation in sera of rabbits affected thereby -- of antibodies to proteus OX_K, which is characteristic Rickettsia tsutsugamushi.)

As several investigators have noted, the most susceptible to infection with Rickettsia tsutsugamushi are white mice (Lewthwaite and Savor, 1936; Blake, 1945; Philip et al, 1946). In our investigations covering several tens of successive passages, a clinical pattern similar to that described in the literature was observed for mice. Upon intra parenteral infection of white mice weighing 12-15 grams with 10 % suspension of the spleen of affected subpassage mice, the animals were observed to succumb chiefly by the 8th-10th (Gilliam strain) and by the 10th-12th day (Karp strain). In 1-2 days before their death, the mice were sluggish, moved little, they evidenced dyspnea, and their fur became ruffled.

Upon dissection of the succumbed and ill mice, we observed injection of vessels of the abdomen, formation in the peritoneal, and sometimes in the pleural cavity of a transparent, mucous, or hemorrhagic exudate, a swelling of the spleen, formation on it of fibrinose incrustation, an increase in the inguinal lymph nodes, and [an increase] in the glycogen storage level of the liver. The quantity of peritoneal exudate amounted to 2 ml. An infective dose of suspension of spleen of subpassage mice for the Gilliam strain amounted to approximately 10⁻⁵ - 10⁻⁶, while a lethal dose was 10⁻³ - 10⁻⁴.

Upon infecting guinea pigs with Rickettsia tsutsugamushi we learned from indications given in the literature that passaging is possible only in animals previously kept under avitaminosis food. In our experiments with such guinea pigs, upon infecting them with 5-10 % of a suspension of ground spleen of subpassage white mice we observed in 3-7 (average 5) days of feverish disease with increase in temperature to 40-41°, kept at the high level for 3-14 (average 6) days. As a result of the infection the guinea pigs almost all succumbed (lytic drop in temperature). A clearly pronounced injection of the vessels of the abdomen, swelling of the spleen, formation on spleen of a fibrinose incrustation, and the accumulation in the peritoneal and pleural cavities of a clear exudate amounting to 10-12 ml was observed. The liver was limp and blood-filled,

and interfocal hemorrhages were noted in the lungs. Further passaging in guinea pigs succeeded in only 2-3 % of the cases, after which infection did not appear.

In culturing in 5-7-day chick embryos at 34°, the Gilliam strain (56-73 passages) induced their death in 100 % of the cases by the 6th-10th day. Hyperemia of the vessels and a small amount of Rickettsia in the gall bladder, chiefly together with signs of lysis, was observed in the perished embryos. The optimal accumulation of Rickettsia was observed in gall bladders of live embryos usually by the 6th-7th day following infection. Profuse accumulation of Rickettsia (++ and +++) was observed in 30-40 % of the embryos. Upon inoculation of 4-6-day embryos of the Karp strain (111-125 passages) the death of 70-80 % of the embryos set in by the 12th-20th day and the accumulation of Rickettsia was scanty. The same pattern had been described by Bengston (1946). However, Smadel et al (1946) in working with this strain observed regular and profuse accumulation of Rickettsia. It is possible that the properties of the Karp strain noted by Bengston and the present authors are due to characteristics of its laboratory variants, exhibiting differing capacity of adaptation to embryos.

In smears taken from the organs of white mice, guinea pigs, and chick embryos Rickettsia was regularly detected. However, in staining the smears, we encountered difficulties recorded in the literature (Fulton and Joyner, 1945; Dierks and Tibbs, 1947). These difficulties were due to the property of Rickettsia tsutsugamushi distinguishing it from other Rickettsia, of losing fuchsin evenly from the surrounding tissues during acid differentiation of the smear. Therefore, the method of Rickettsia staining according to Zdrobowskiy, widespread in Soviet laboratories, has proven inapplicable. After testing many methods of staining, we concluded that the best of the methods available to us is staining according to Romanovskiy-Gizme and according to Fulton and Joyner (1945). In Rickettsia tsutsugamushi preparations stained by these methods polaroy-stained diploforms were found, being midway in shape between diplococci and diplobacilli. By size, they were somewhat larger than Rickettsia burneti and smaller than the Rickettsia of the tick-borne spotted fever group. In a smear made of live chick embryo yolk sac, dissected on the 6th-7th day following infection Rickettsia tsutsugamushi appeared as a monomorphous culture, which differentiated it from Rickettsia of other species inclined to polymorphism (Figure 1). A similar observation has been recorded by Shteynkhaiz (1950). Sometimes it was possible to observe the formation of chainlets. In smears made of tissues and peritoneal and pleural exudates of white mice and guinea pigs, Rickettsia tsutsugamushi was detected in large amount inside and outside of cells. Rickettsia for the most part lay alongside the nucleus or in the form of small clusters by the periphery of the cell (Figure 2 and 3).

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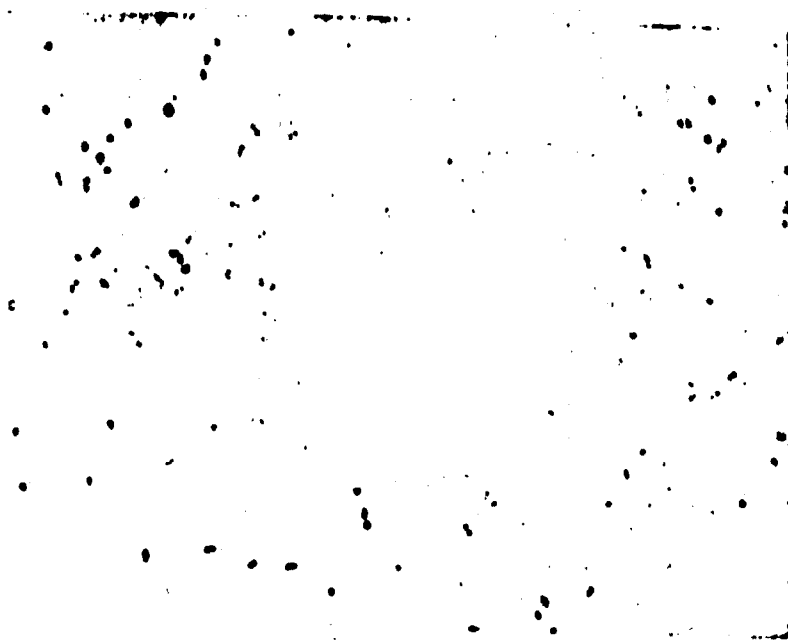


Figure 1. *R. tsutsugamushi* in smear taken from tissues of gall bladder of chick embryo. Stained after Romanovskiy - Gimze. X 1000.

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Figure 2. *R. tsutsugamushi* in smear of peritoneal exudate of guinea pig. Stained according to Romanovskiy - Gimze. X 1000.

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Figure 3. R. tsutsugamushi in smear of white mouse peritoneal exudate. Staining according to Zdrobovskiy. X 1000.

We traced the dynamics of *Rickettsia* accumulation and its distribution in several organs of white mice. A knowledge of these facts is necessary for the timely carrying out of passages and preparation of antigen from parenchymatose organs and thus far has not been found in the literature available to us.

Male white mice weighing 12-15 grams were infected intraperitoneally with a 20 % solution of ground spleens of affected mice, sacrificed on the 7th-8th day following infection with the Gilliam strain. In the period from the second to the thirteenth day following infection, five mice were dissected daily and biopsy smears were prepared from the abdomen, impressions from the spleen, liver, kidneys, lungs, and preparations of peritoneal and pleural exudates. The Nikiforov mixture was used for fixation; smears were stained according to Romanovskiy-Gimze. In smears made on the second-fourth day following infection, *Rickettsia* was not found. Beginning from the 5th day formation of peritoneal exudate, considerable swelling of spleen, and presence of *Rickettsia* were noted in outwardly healthy mice. In smears of abdomen material, *Rickettsia* appeared individually and in small groups in mesothelial cells. By the 6th day, the mice became sluggish. Upon dissection, considerable exudate was noted in them, together with profuse accumulation of *Rickettsia* in the exudate and abdominal cells, signs of *Rickettsia* in all the spleens of the animals, and in certain mice -- in the liver.

By the 7th day, some of the experimental mice had succumbed, all others evidenced clinical signs of the disease: sluggishness, dyspnea, rumpling of fur. In smears made of abdomens, peritoneal exudate, spleen, liver, kidneys, and lungs, *Rickettsia* located as individualized microorganisms and in groups within and without cells were detected. On the 8th day, the mice continued to die. In the live mice dissected, profuse accumulation of *Rickettsia* was established in smears of abdomen, spleen, liver, kidneys, lungs, and peritoneal exudate. On the 9th day, pleural exudate with a small amount of cellular elements, but with profuse *Rickettsia* content, located extracellularly and in part within cells appeared. By the 9th-13th day, abundant accumulation of *Rickettsia* was noted in all these organs, although in many preparations signs of lysis began to appear: the contours of the *Rickettsia* became diffuse, sometimes swollen, and the centrodesmos between the polar formations was elongated. Observations were not conducted past the 13th day because of the death of all the experimental mice.

Based on the morphological characteristics of the infectious process described it is clear that it is a typical example of the septic form of peritoneal rickettsiosis (Zdrodovskiy and Golinevich, 1956) characterized by rapid onset of generalization of infection, profuse accumulation of *Rickettsia* in different organs and by the death of the animals.

Conclusions

1. Well-known data from the literature is confirmed to the effect that white mice are highly susceptible and sensitive to infection with *Rickettsia tsutsugamushi*: the death of 90-100 % of the mice was observed by the 8th-13th day upon infection with the Gilliam strain and by the 12th-15th day upon infection with the Karp strain.

2. Upon infection with the Gilliam strains, *Rickettsia* was detected in the organs of animals from the 5th day to the day of death, profuse accumulation of *Rickettsia* in the spleen, liver, lungs, peritoneal, and pleural exudate, was observed from the 8th to the 13th day. (

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